A

SYNOPSIS

On

Green Synthesis, Characterization and Applications of Some Metal Nanoparticles, using Plant Extracts available in India

For the Admission in Doctor of Philosophy

Submitted by

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Ph.D. Synopsis

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Introduction

Nanoscience is a vast field of material science which deals with the study of materials at nano scale, the size ranging between 1 to 100 nm and may be 0, 1, 2 or 3 Dimensional. When the size of the material reduces to nanoscale, it exhibits different properties than the same material in bulk form. The factors such as Enhanced surface to volume ratio, Quantum confinement effect, Enhanced bonding characteristics and Enhanced Permeability Retention (Metal NPs) etc come into picture at nano scale.

There are basically two methods of synthesizing Nanoparticles.
Top Down Approach And Bottom up Approach
Top Down Approach is "Breaking into smaller one by physical forces" and includes Chemical Etching, Ball Milling, Electron Explosion, Laser Ablation methods. Bottom Up Approach is "Self combining mechanism by chemical forces" & includes Chemical Vapour Deposition, Sol gel process, Atomic Condensation, Solution Combustion Synthesis (SCS), Self Propagating High Temp Synthesis (SHS) and *Green Synthesis.*

Green Synthesis is the method of synthesizing Nanoparticles in which reduction of metal salt are carried out by using non-toxic safe reagents. The reduction process of metal salts is carried out by the '*Biogenic'* reducing agents like plants and microorganisms (bacteria, algae, fungi, etc.). The non-biological methods (chemical and physical) that are used in the synthesis of nanoparticles, have a serious hazardous and high toxicity impact on living organisms.
Recently, many studies have proved that the plant extracts act as a potential precursor for the nanoparticles synthesis in non-hazardous ways. Since the plant extract contains various secondary metabolites, it acts as reducing and stabilizing agents for the bioreduction reaction to synthesize novel metallic nanoparticles.

There are several advantages of Green Synthesis:- It is *One Step One Pot* process consisting of reducing as well as Stabilizing agent altogether. It is an Eco-Friendly, non-toxic method. In this process, the external experimental conditions like high energy, pressure, temperature are not required.

Aims and Objectives

Plants are better for the biosynthesis of nanoparticles among all biological organisms because microbes (Bacteria, Fungi etc) require relatively higher incubation time for the reduction process.

- Under the proposed work our aim is to achieve Green Synthesis of metal nanoparticles of Copper and Silver, using plant extracts well found in Indian habitat (like Tulsi, Nutmeg, Cloves, Marie Gold, etc). The method will be elaborated and standardized, to have a simple & non-toxic approach of obtaining metal NPs.

- The Characterization of metal nanoparticles using UV-Spectroscopy, AFM, XRD, SEM and etc. characterizing tools, will be done to analyze the particle size, texture, uniformity and surface area.

- Discussion of its Applications & future aspects is proposed to be done by investigating the photocatalytic, antimicrobial or other properties of synthesized NPs.

Literature Review

The rich biodiversity and easy availability of plant entities have been highly explored for the nanomaterials synthesis. Recently, the biosynthesis of nanosized particles, from plant leaves ,flowers, tubes have been reported successfully.

There are variety of plants, such as Brassica Jucea (Indian Mustard), Medicago sativa (Alfalfa), Heliantus annus (Sunflower), Ocium Sanctum (Tulsi), T. Arjuna (Arjuna Tree), Syzygium aromaticum (Cloves), etc. which have been used for the synthesis of gold, silver, nickel, cobalt, zinc and copper nanoparticles[1].The molecules and phytochemicals, in the biosynthesis of nanoparticles, have been identified through infrared spectroscopic studies.

Green Synthesis of silver nanoparticles from Ocium sanctum leaf (Tulsi) extracts has been done, in which bio extract is used as reducing agent to reduce silver ions formed

from precursors to silver nanoparticles[2]. When titration dropwise addition of leaf extract with silver nitrate and simultaneously mixing on magnetic stirrer is done, it immediately reduces into silver nanoparticles _ From Yellow to Dark Brown Colour.
The Color change indicates the completion percentage of Redox Reaction.
Then centrifugation at 4000rpm for 15 min at room temp is done. The pellet obtained is dried and powdered.

Copper Nanoparticle Synthesis using Sygzium Aromoaticum (Cloves) have been reported[3]. A phytochemical Eugenol is found in cloves which is a significant reducing agent.
Cloves are washed by DDW and heated at 70-80 degree Celsius in 10 ml sterile water and then filtered. For the reduction of Cu ions, 5 ml of filtered cloves extract was mixed to 5 ml of freshly prepared 0.001M aqueous of $CuSO_4$ solution. It was then kept for incubation for 1hr. Within a particular time, the change in colour was noted from Dark Brown to Sea Green. Thus Color Change indicates reduction and reduced copper nanoparticles were obtained.

Copper Nanoparticles using T. Arjuna Bark extract- Freshly obtained T. Arjuna tree bark[4] is washed with DDW and heated with Sterilzed distilled water for 5 minutes, to extract the phytochemical. Then it is mixed with cuper Nitrate aqueous solution and is heated at oven. Intially the mixture is pale yellow and after 8 minutes the mixture is dark brown, showing the 100% reduction in 8 minutes. Biomolecules in Arjuna Bark are responsible for the process.
Here, it has been observed that Micro oven heating increases the absorption process in the reaction.

Mahogany leaf extract contains various polyhydroxy limonoids which are responsible for the reduction of Au(III) and Ag(I) ions leading to the formation and stabilization of Au and Ag nanopaticles[5]. When exposed to aqueous mahogany leaf extract, competitive reduction of Au(III) and Ag(I) ions present simultaneously in same solution leads to the production of bimetallic Au/Ag alloy nanoparticles. UV-visible spectroscopy was used to monitor the kinetics of nanoparticles formation. UV-visible spectroscopic data and TEM images revealed the formation of bimetallic Au/Ag alloy nanoparticles.

Green Synthesis of copper nanoparticles using leaf extract of Ageratum houstanianum Mill. (Blue Mink plant)- *Ageratum Houstanum* is an annual herb and commonly known as Foss Flower or Blue Mink. The research was undertaken for the first time with the objective to synthesize CuNPs from the leaf extract of AH[6] and characterization of phytosynthesized AH-CuNPs through spectroscopic and microscopic techniques.
Precursor used $CuCl_2$ and Ageratum Houstanianum leaf extract was used as reducing agent.
Through various tests (Mayers Test, Ferric chloride Test, etc) diagnosis of the phytochemicals presenct in AHLE was done. Alkoids, Flavoids, Steroids, etc phytochemicals were found.

Aromal and D. Philip achieved Green synthesis of gold nanoparticles using Trigonella foenum-graecum (Methi) and its size dependent catalytic activity[7]. Plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticles formation. In some reports the biosynthesized nanoparticle effectively controlled oxidative stress, genotoxicity and apoptosis related changes.

With the emergence and increase of microbial organisms resistant to multiple antibiotics, the antimicrobial effects of silver (Ag) nanoparticles was investigated against yeast, Escherichia coli and Staphylococcus aureus[8]. In these tests, Muller Hinton agar plants were used and Ag nanoparticles of various concentrations were supplemented in liquid systems.

In addition to these work, synthesis of silver nanoparticles using Rhizophora Apiculta (a mangrove species) and glucose[9] have been done and comparison of antimicrobial efficacy of the silver nanoparticles of biological and chemical have been analysed.

Materials & Methodology

Although large numbers of synthesis methods are reported for the metal NPs, the literature shows that biological or green methods using plant extracts are relatively new approach.

Plants have potential to store heavy metals. They have phytochemicals (Like Flavones, Terpenoids, Sugar, Ketones, Aldehydes, Carboxylic Acid, Amino Groups), which can reduce metal salts in Nanoparticles.

Materials

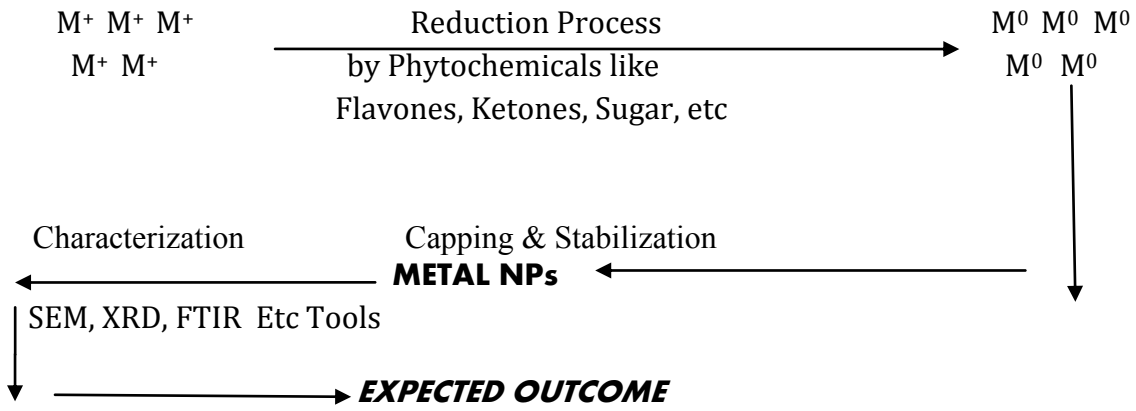
- We shall use metal Salts of Silver (Silver Nitrate $AgNO_3$), Copper (Copper Nitrate $Cu(NO_3)_2$, Copper Sulphate $CuSO_4$, Iron ($FeCl_3$) etc.
- Plants Extracts of the species easily found in Indian habitat like Tulsi, Clove, sorghum, Nutmeg, Cinnamon bark , Marie Gold etc will be used for bio synthesis.
- Common Sterilized filtration equipments (such as Flask, Micro oven) & Materials like DDW, Whatman's No. 1 Filter etc. type general laboratory equipments are required.

Methodology

Metal salt solutions of required concentration will be prepared in the distilled water. A fixed volume of this solution will be taken in the flask and a fixed volume of prepared plant extract will then be added dropwise to it with constant stirring and heating at standard parameters.

The reduction reaction will be monitored by colour change of solution.

The process can be summed up by the following flow chart as :-



Characterization

Nanoparticles are generally characterized by investigating their size, shape and surface area. So, the Characterization of the bio-synthesized Nanoparticles is done by :-

UV-spectroscopy – It is a technique used to quantify the light that is absorbed and scattered by a sample. It may be used to analyze size and concentration dependent optical properties of synthesized NPs.
Basic Working Principle– The data is plotted as scattering intensity through synthesized sample, as a function of wavelength of light used.

Atomic Force Microscopy (AFM) –It is a versatile and powerful microscopy technology that provides image of nanomaterials in three-dimensional topography.
Basic Working Principle- The data is collected by piezoelectric effect of the synthesized sample that facilitate accurate and precise movement on electronic command enabling precise scanning.

Scanning Electron Microscopy (SEM) shall be employed for studying face texture or microstructure.
Basic Working Principle- An electron beam is used to obtain images of the synthesized samples with a resolution down to the nanometer scale.

X-Ray Diffraction (XRD) pattern , the most widely used characterization tool can be used to characterize the structure, nature and lattice parameters and crystalline grain size.
Basic Working Principle – Synthesized sample is irradiated with incident X-rays and intensities and scattering angle of the X-rays that leaves the material is measured.
It uses Bragg's Law stating - $\mathbf{n\lambda = 2\,d\sin\theta}$ where, d is interplanar spacing, λ is wavelength of X-ray, θ is angle of diffraction and n is integer.

Fourier's Transformation Infra Red (**FTIR)**- Involvement of Phytochemicals in re-stabilization and reduction of nanoparticles may be analyzed by FTIR Spectroscopy.
Basic Working Principle –The sample's absorbance of the infrared light's energy at various wavelength is measured and IR spectrum graph is plotted to determine the material's molecular composition and structure.

Relevance of Proposed Work

Nanoparticles appear to be bright and promising materials in the field of medicine, environment, industrial, daily uses, etc because of its rapid, economical and eco-friendly procedure. The problems of handling hazardous gaseous residues in other methods (SCS, SHS) could be avoided. As the process is "one-step, one-pot" Process and is carried out at ambient temperature and pressure and also near Neutral pH.

Future research and development of prospective "Green" material/Nanoparticles synthesis will be enhanced at laboratory bases work to an industrial scale.
Also, the BioSynthesis of metals by many plant species and their applications is still unexplored. So, ample possibilities remain for the exploration of new green synthesizing strategies based on its biogenic synthesis.
Small and smaller Transistors and Switches in Electronics, Energy Storage Devices, nano medicines and nanorobots in treatment and drug delivery, Nanoparticles use in Clothing and daily applications such as anti microbial techniques, strength increment of sports articles by adding nanotubes, cost efficient sensors etc;- These type of various utilizations are owed to Nanotechnology. So, there are many opportunities to use biological systems for nanoparticle synthesis.

Expected Outcomes

- The existing method of green synthesis of metal nanoparticles will be standardized.
- The Green Method adopted, will promote the eco-friendly approach of synthesizing NPs. The plants of Indian habitat may be utilized for a cost efficient & easily available route of green synthesis in India, with limited instrumental resources.
- The result of this research work is expected to be explained reflecting the advancement in the area of Green Synthesis of NPs.
- The Characterization, Application and Analysis of obtained Metal NPs may be utilized for antimicrobial, catalytic or other activities.

Chapterization of Proposed Ph.D Thesis

Tentatively, the proposed research work on "Green synthesis, characterization & applications of some metal nanoparticles, using plant extracts" will run in five chapters. Under this work, we will synthesize metal nanoparticles of Copper, Silver and/or Iron using plant extracts available in India.

CHAPTER-I will start from the outline of the research work.. There will be brief discussion regarding NPs, biological methods and plants extracts. It will include an introduction with the theme of the topic, its importance and objectives etc

CHAPTER-II will contain review of literature, previous and existing state of knowledge on the topic of research. Review of literature will provide analysis of all current information relevant to the topic.

CHAPTER-III will deal with the experimental details on material used, synthesis methodology and tools and techniques for characterization of synthesized nano-materials.

CHAPTER-IV will include detailed discussion on results of present work which includes the key points and various aspects of synthesis.

CHAPTER-V, will summarize and conclude the proposed research work. The future scope of the work and applications will also be discussed under this chapter.

Reference will be given at the end of thesis.

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